

## ARCEUTHOBIMUM RUBRUM (VISCACEAE) IN MEXICO

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### ABSTRACT

*Arceuthobium rubrum* (Viscaceae) is a distinctive species of dwarf mistletoe having red to reddish-brown plants and red, shiny fruits. It is primarily distributed in the Sierra Madre Occidental of Durango, Mexico, but in 1972 a population of *A. rubrum* was reported from Oaxaca, Mexico, more than 1000 km south of the nearest population in southern Durango. Initially, this population was classified as a disjunct population of *A. rubrum*, but in 1989 it was described as a new species: *A. oaxacacum*. However, our morphological measurements and observations of the phenology for plants from Durango and Oaxaca indicate these populations are morphologically similar and flower and disperse seed at approximately the same time, supporting the results of recent molecular analyses indicating that the Oaxacan populations represent disjunct populations of *A. rubrum*.

### RESUMEN

*Arceuthobium rubrum* (Viscaceae) es una especie distintiva de muérdago enano de color rojo a café rojizo y con frutos rojo brillantes. Se distribuye principalmente en la Sierra Madre Occidental de Durango, México, pero en 1972 se reportó una población para Oaxaca, México, a más de 1000 km al sur de la población más cercana al sur de Durango. La población de Oaxaca fue clasificada inicialmente como una población disyunta de *A. rubrum*, pero en 1989 fue descrita como una nueva especie: *A. oaxacacum*. Sin embargo, nuestras mediciones morfológicas y observaciones de la fenología de las plantas de Durango y Oaxaca indican que esas poblaciones son morfológicamente similares y florecen y dispersan las semillas aproximadamente al mismo tiempo, lo cual apoya los resultados de recientes análisis moleculares que indican que las poblaciones de Oaxaca representan poblaciones disyuntas de *A. rubrum*.

Key Words: *Arceuthobium oaxacacum*, *Arceuthobium rubrum*, México, morphology, phenology.

*Arceuthobium rubrum* Hawksw. & Wiens (Rubiaceae) is a distinct species with reddish to brownish-red plants and shiny fruits and is a common parasite of several pines (*Pinus* spp., Pinaceae, Table 1) in northwestern Mexico (Hawksworth and Wiens, 1965, 1972, 1996; Mathiasen et al. 2008). When a dwarf mistletoe with brownish red plants and shiny, red fruits was discovered in western Oaxaca on *Pinus pseudostrobus* Lindley by Dr. Roger Peterson in 1972, Hawksworth and Wiens (1977) classified it as a disjunct population of *A. rubrum*. However, based on further studies of two populations of *A. rubrum* in Oaxaca, Hawksworth and Wiens concluded that these populations were sufficiently distinct from those in Durango to describe the Oaxaca populations as a new species: *A. oaxacacum* Hawksw. & Wiens (Hawksworth and Wiens 1989). They separated *A. oaxacacum* from *A. rubrum* based primarily on differences in plant size, plant color, length of pistillate spikes,

branching angle of staminate and pistillate spikes, and the tendency to form systemic infections or not (Hawksworth and Wiens 1989). Host range was also mentioned as a possible distinction between these taxa, although the hosts of *A. oaxacacum* do not occur in the range of *A. rubrum* (Table 1).

Comparisons of *A. rubrum* and *A. oaxacacum* have been difficult because Hawksworth and Wiens (1989) did not include a description of the flowers of *A. oaxacacum* when they described it and only included information on the mean length of fruits (3.5 mm) in their Latin description of this species. Furthermore, in their revised monograph of *Arceuthobium*, Hawksworth and Wiens (1996) did not include any information on the characteristics of the flowers or fruits of *A. oaxacacum*. More recently, Nickrent et al. (2004) reported that *A. rubrum* and *A. oaxacacum* could not be distinguished using internal transcribed spacer DNA and chloroplast *trnL* DNA sequenc-

TABLE 1. HOSTS OF *ARCEUTHOBIMUM RUBRUM* IN DURANGO AND OAXACA FROM HAWKSWORTH AND WIENS (1989, 1996). <sup>1</sup>Host susceptibility class is defined in Hawksworth and Wiens (1996). <sup>2</sup>Hosts of *Arceuthobium oaxacanum* from Hawksworth and Wiens (1989, 1996). <sup>3</sup>Does not occur in Oaxaca (Perry, 1991; Farjon and Styles, 1997). <sup>4</sup>Does not occur in Durango (Perry, 1991; Farjon and Styles, 1997). <sup>5</sup>Distributed in both Durango and Oaxaca, but did not occur in stands infested by *A. rubrum* in Oaxaca.

Host susceptibility class <sup>1</sup>	Durango	Oaxaca <sup>2</sup>
Principal	<i>Pinus cooperi</i> <sup>3</sup>	<i>Pinus lawsonii</i> <sup>4</sup>
	<i>P. durangensis</i> <sup>3</sup>	<i>P. michoacana</i> <sup>4</sup>
	<i>P. engelmannii</i> <sup>3</sup>	<i>P. pseudostrabus</i> <sup>4</sup>
	<i>P. herrerae</i> <sup>3</sup>	
	<i>P. teocote</i> <sup>5</sup>	
Occasional		<i>P. oaxacana</i> <sup>4</sup>

es. Therefore, in 2007, we began studies of the Durango and Oaxaca populations of *A. rubrum* in order to gather additional morphological data for their flowers, fruits, and seeds. This allowed the flower and fruit characteristics of *A. rubrum* and *A. oaxacanum* to be compared for the first time. We also made additional observations of the phenology of both species starting in 1999.

METHODS

Morphological Measurements

Specimens from two of the three currently known populations of *A. oaxacanum* were sampled in 2007 and five populations of *A. rubrum* were sampled in 2008 (Fig. 1). These populations included the type localities for *A. rubrum* and *A. oaxacanum* (Hawksworth and

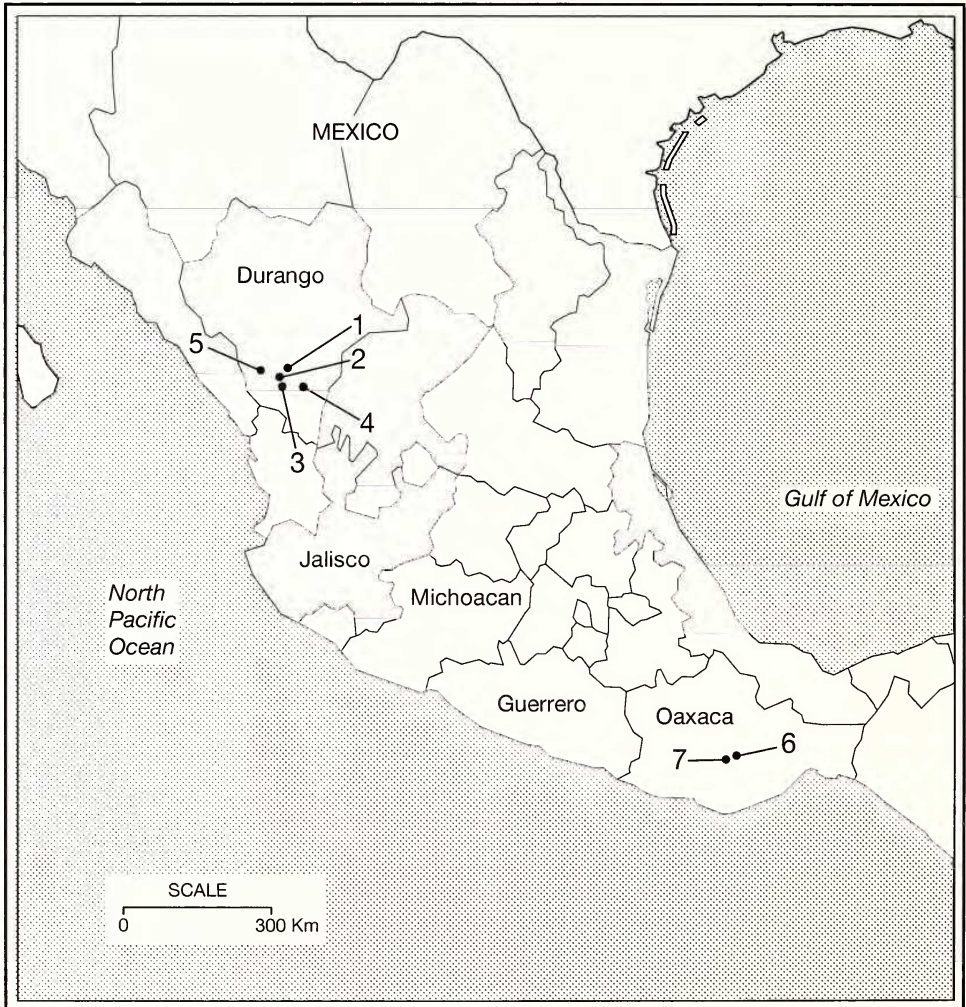


FIG. 1. Approximate locations of populations of *A. rubrum* sampled in Durango and Oaxaca, Mexico. Sites are listed under specimens examined; site 1 is the type locality for *A. rubrum* and site 6 the type locality for *A. oaxacanum*.

Wiens 1965, 1989; Fig. 1). For each population, 20–30 non-systemically infected branches with mature plants (10–15 males and 10–15 females) were collected. The largest dwarf mistletoe plant from each infection was measured; plant measurements were made within 24 hrs of collection. Measurements for flowers and fruits/seeds were completed in July or August when flowers were open and fruits were mature. The following morphological characters were measured: 1) plant height, basal diameter, third internode length and width, and color; 2) mature fruit length, width, and color; 3) seed length, width and color; 4) staminate flower diameter for flowers with 3 perianth lobes; 5) number, color of the adaxial surface, and length and width of staminate perianth lobes; 6) anther distance from the perianth lobe tip; and 7) anther diameter. A total of 50 measurements were made for each of these characters. Furthermore, we only measured plants from non-systemic infections for both species, because plants from systemic infections tend to be smaller than those from non-systemic infections (Hawksworth and Wiens 1996). A one-way analysis of variance (ANOVA,  $P \leq 0.05$ ) was used to determine if there were statistical differences between the means of the morphological characters measured.

*Specimens examined.* MEXICO. DURANGO. 47 km E of El Salto on Mexico Rte. 40, on *Pinus teocote*, 2 Aug 2008, *Mathiasen 0808* (ASC) (site 1, Fig. 1, type locality of *A. rubrum*); 6.5 km S of Mexico Rte. 40 on rd to Regocijo, on *Pinus teocote*, 31 Jul 2008, *Mathiasen 0813* (ASC) (site 2, Fig. 1); 12.6 km S of Mexico Rte. 40 on rd to Regocijo, on *Pinus teocote*, 31 Jul 2008, *Mathiasen 0816* (ASC) (site 3, Fig. 1); 51 km S of Durango on rd. to La Flor, on *Pinus teocote*, 30 Jul 2008, *Mathiasen 0811* (ASC) (site 4, Fig. 1); 5 km W of El Salto on Mexico Rte. 40, on *Pinus teocote*, 29 Jul 2008, *Mathiasen 0809* (ASC) (site 5, Fig. 1). OAXACA. 13 km S of Miahuatlan on Mexico Rte. 175, on *Pinus lawsonii*, 22 Jul 2007, *Mathiasen 0727* (ASC), and 24 Jul 2007, *Mathiasen 0729* (ASC) (site 6, Fig. 1, type locality of *A. oaxacacum*); 7 km W of Santo Tomas Tamazulapan, on *Pinus lawsonii*, 22 Jul 2007, *Mathiasen 0726* (ASC) (site 7, Fig. 1).

#### Phenology

Observations of flowering and seed dispersal in Durango were made in December 1999, March 2003, March 2004, July 2005, March 2007, September and October 2007, and July 2008. Observations in Oaxaca were made in December 2000, September 2004, September 2006, and July 2007.

## RESULTS

### Morphological Measurements

The means and ranges for plant heights, basal diameters, and third internode dimensions of the populations we sampled in Oaxaca and Durango were nearly identical (Table 2). Male plants were slightly larger than female plants from both regions, but approximately the same for each sex. Only the length of the third internode of male plants from Oaxaca was significantly different than the length of this internode for male plants from Durango. Hawksworth and Wiens (1989, 1996) reported that the mean height of *A. rubrum* plants (male and female combined) from Durango was approximately 10 cm and those from Oaxaca were approximately 12 cm. When we combined male and female plant height data, the mean heights of plants from both areas were approximately 12 cm (Table 2). The ranges in plant heights Hawksworth and Wiens (1989) reported were approximately the same; 8–18 and 8–20 cm for plants from Durango and Oaxaca, respectively. The plant height ranges we recorded were only slightly different than those reported by Hawksworth and Wiens, but we also found that the range in plant heights was approximately the same in both regions.

The mean widths for staminate spikes were also similar between the populations in Oaxaca and Durango, but the length of staminate spikes was significantly different (Table 3). Although the means for the flower characters measured were slightly larger for the flowers from male plants in Oaxaca, the means were not significantly different. The flower diameters for 3-merous flowers were smaller than most other species of

TABLE 2. PLANT HEIGHTS, BASAL DIAMETERS, AND THIRD INTERNODE MEASUREMENTS FOR *ARCEUTHOBIUM RUBRUM* IN DURANGO AND OAXACA. Measurements are expressed as mean (standard deviation) values. Mean values between the two locations are not significantly different except for the third internode length in males (ANOVA,  $P \leq 0.05$ ).

Character	Durango	Oaxaca
Plant height (cm)		
Male	14.2 (3.9)	13.9 (3.7)
Female	10.3 (2.5)	10.0 (2.7)
All plants (cm)	12.3 (3.5)	11.9 (3.7)
Basal diameter (mm)		
Male	3.7 (1.0)	3.5 (0.6)
Female	4.0 (1.0)	3.8 (1.0)
Third internode length (mm)		
Male	10.9 (3.5)	9.8 (3.2)
Female	9.1 (2.3)	8.8 (2.3)
Third internode width (mm)		
Male	2.8 (0.6)	2.7 (0.6)
Female	3.0 (0.6)	2.9 (0.6)

*Arceuthobium* (Hawksworth and Wiens 1996), averaging only 1.6 and 1.7 mm in Durango and Oaxaca, respectively. Another unusual characteristic of the flowers of *A. rubrum* reported by Hawksworth and Wiens (1965, 1996) is that it had flowers that were barely open, even during their peak of anthesis. We also observed this characteristic in Durango, but some flowers did open to the extent that they could be easily measured. Our observations in Oaxaca indicated the flowers there also do not open completely during anthesis.

Fruit and seed dimensions demonstrated the greatest difference between the mistletoe populations in Oaxaca and Durango, but differences were only 0.1–0.2 mm larger for the populations in Oaxaca and were not significantly different (Table 3). Although Hawksworth and Wiens (1989, 1996) reported that the mean fruit length in Oaxaca and Durango was about 3.5 mm, none of the mature fruits we measured in either state were that small. The mean lengths of mature fruits we measured in Oaxaca and Durango were nearly one mm larger than reported by Hawksworth and Wiens (4.4 and 4.3 mm, respectively). We measured mature fruit as large as 5 mm in Durango and 5.2 mm in Oaxaca.

Plant color for male and female plants was similar for populations from Oaxaca and Durango also. The predominant color of male and female plants in Durango and Oaxaca was reddish-brown (76%, and 70%, respectively). Other plants were either red or brown, but a few female plants in Durango were very dark; nearly black in color. Plants from both regions that were red when fresh dried to a dull brown.

Another distinction between the Oaxaca and Durango populations noted by Hawksworth and Wiens (1989, 1996) was that the dwarf mistletoe typically induced systemic infections on its pine hosts in Oaxaca, while those in Durango typically

did not. However, our observations in Oaxaca indicated that the mistletoe also commonly formed non-systemic infections there. In Durango, we observed that *A. rubrum* frequently induced systemic infections, particularly on *Pinus teocote* Schiede ex Schltdl. & Cham. and *Pinus durangensis* Martínez. Therefore, the infrequency of non-systemic infections in Oaxaca versus systemic infections in Durango does not appear to be a consistent difference between these populations. Furthermore, we did not observe any distinctive differences in the size of the witches' brooms caused by these dwarf mistletoes on their pine hosts in Oaxaca versus Durango, although Hawksworth and Wiens (1989, 1996) reported that the mistletoe in Oaxaca induced larger brooms on its hosts.

Hawksworth and Wiens (1989) also justified the classification of the populations in Oaxaca as a distinct species because plants there had spikes that branched at nearly right angles (90°) from the main axis of plants while the spikes of plants in Durango usually branched at angles of about 45°. We observed that younger plants in both areas tended to have spikes that branched at about 45° and older plants had more spikes that branched at nearly right angles to the main axis of the plant. In addition, the young staminate spikes near the top of older male plants from both regions tended to branch at about 45° and older spikes commonly branched at right angles. However, these branching patterns varied widely between different populations. Therefore, the branching angle of spikes was not a consistent difference between the populations in Durango and Oaxaca.

The *A. rubrum* populations near Altares, Durango have been reported to have plants that are much larger than those in other areas of Durango (Hawksworth and Wiens 1996). We examined two populations in July 2008 that Hawksworth and Wiens (1996) classified as *A. rubrum* near Altares and classified these as *Arceuthobium vaginatum* (Willd.) Presl subsp. *vaginatum*. Male and female plants of these populations were very large, dark brown to nearly black, and had much larger basal diameters (>1 cm) than the *A. rubrum* plants we examined elsewhere in Durango. Although we also noted that some of the fruits of these populations were shiny, they were not dispersing seed as were populations of *A. rubrum* we examined in Durango at that time. Furthermore, male plants of the Altares populations were not flowering in July 2008. They appeared to have already flowered earlier in the year. Therefore, the phenology of the Altares populations also supports their classification as *A. vaginatum* subsp. *vaginatum* because this taxon flowers in March–April and disperses seed in August through September (Hawksworth and Wiens

TABLE 3. STAMINATE SPIKE, FLOWER, FRUIT, AND SEED MEASUREMENTS FOR *ARCEUTHOBIMUM RUBRUM* IN DURANGO AND OAXACA. Measurements are expressed as mean (standard deviation) values. Mean values between the two locations are not significantly different except for staminate spike length (ANOVA,  $P \leq 0.05$ ).

Character	Durango	Oaxaca
Staminate spike length (mm)	11.4 (2.4)	10.8 (3.4)
Staminate spike width (mm)	1.5 (0.1)	1.6 (0.2)
Staminate flower diameter (mm)	1.6 (0.2)	1.7 (0.2)
Perianth lobe length (mm)	0.7 (0.06)	0.8 (0.06)
Perianth lobe width (mm)	0.7 (0.10)	0.8 (0.14)
Anther diameter (mm)	0.3 (0.08)	0.4 (0.08)
Mean fruit length (mm)	4.3 (0.3)	4.4 (0.4)
Mean fruit width (mm)	2.8 (0.2)	3.0 (0.3)
Mean seed length (mm)	2.4 (0.2)	2.5 (0.3)
Mean seed width (mm)	1.2 (0.09)	1.3 (0.13)

1996). Although the Altares populations Hawksworth and Wiens' classified as *A. rubrum* were parasitizing *Pinus arizonica* Engelm., they never classified this pine as a host of *A. rubrum* (Hawksworth and Wiens 1996, see pages 49 and 364).

### Phenology

Male plants in Durango began flowering in early July and flowering peaked from mid to late July, but some plants continued to flower through August into early September. Male plants in Oaxaca began flowering at the same time, but some plants were also observed flowering in mid September. Therefore, the time of anthesis is nearly the same for these dwarf mistletoe populations, but extends slightly longer in Oaxaca. Seed dispersal started in mid July in Durango and continued into September, with its peak in late August to early September. Seed dispersal in Oaxaca occurred slightly later based on observations in September 2007. Female plants in Oaxaca were just starting to disperse seed in mid September that year, so seed dispersal peaked in late September and continued into October. The slightly later periods for flowering and seed dispersal for the Oaxaca populations may be due to their more southern distribution or that they occurred at lower elevations (1760–2100 m) than the populations in Durango (2300–2600 m).

### DISCUSSION

Our morphological data and observations of the phenology of the *A. rubrum* populations from Oaxaca and Durango supported the classification of these widely separated populations as one species, as was concluded earlier by Hawksworth and Wiens (1977) and supported with molecular analyses by Nickrent et al. (2004). Therefore, we recommend that the dwarf mistletoe populations with reddish brown plants in Oaxaca be treated as extremely disjunct populations of *A. rubrum*. Because of the similarity between the male and female plants, flowers, and fruits of the Oaxaca and Durango populations of *A. rubrum*, the classification of these populations as different species is not warranted at this time.

The only host of *A. rubrum* that occurs in both Durango and Oaxaca is *Pinus teocote* (Perry 1991; Farjon and Styles 1997), but this pine does not occur in the *A. rubrum* populations we sampled in Oaxaca (Table 1). Therefore, host susceptibility based on natural infection of pines in Oaxaca and Durango could not provide information on the taxonomic relationship of

these populations (Hawksworth and Wiens 1989). Although the pine hosts of *A. rubrum* in Oaxaca are not closely related to its pine hosts in Durango (Farjon and Styles 1997), this is insufficient evidence to support the classification of the Oaxaca populations as a different species.

The large geographic separation of the *A. rubrum* populations in Oaxaca from those in Durango (ca. 1200 km) certainly suggests that these populations could have developed characters that support separate taxonomic status. Although some of the characters we measured were slightly larger for the populations of *A. rubrum* we sampled in Oaxaca than those in Durango, we do not feel the differences were large enough to support the recognition of the Oaxaca populations as a different species or subspecies. If additional populations of *A. rubrum* were to be found in the large geographic gap between Oaxaca and Durango, it would lend further support to our interpretation of the widely separated populations of *A. rubrum* being conspecific.

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